Crystal Structures of Giardia Lamblia Guanine Phosphoribosyltransferase at 1.75 Å and Other Phosphoryl Transfer Reactions

W. Shi, C. Li, V. Schramm, S. Almo, D. Lawrence, X.-L. Zhang, and J. Condeelis (Albert Einstein College of Medicine) N. Munagala and C. Wang (U. of California) P. Tyler and R. Furneaux (Industrial Research Ltd) and C. Grubmeyer (Temple U.)

Abstract No. shi2753
Beamline(s): **X9B**

Giardia lamblia, the protozoan parasite responsible for giardiasis, requires purine salvage from its host for RNA and DNA synthesis. G. lamblia expresses an unusual purine phosphoribosyltransferase with a high specificity for guanine (GPRTase). The enzyme's sequence significantly diverges from those of related enzymes in other organisms. The transition state analogue immucillinGP is a powerful inhibitor of HGXPRTase from malaria [Li, C.M., *et al*. (1999) Nat. Struct. Biol. 6, 582-587] and is also a 10 nM inhibitor of G. lamblia GPRTase. Cocrystallization of GPRTase with immucillinGP led unexpectedly to a GPRTase.immucillinG binary complex with an open catalytic site loop. Diffusion of ligands into preformed crystals gave GPRTase.immucillinGP.Mg(2+) pyrophosphate complex in which the open loop is stabilized by crystal contacts. These structures were solved by multiple anomalous dispersion (MAD) methods using seleno-methionyl substituted protein. G. lamblia GPRTase exhibits substantial structural differences from known purine phosphoribosyltransferases at positions remote from the catalytic site, but conserves most contacts to the bound inhibitor. The filled catalytic site with an open catalytic loop provides insight into ligand binding. One active site Mg(2+) ion is chelated to pyrophosphate, but the other is chelated to two conserved catalytic site carboxylates, suggesting a role for these amino acids. This arrangement of Mg(2+) and pyrophosphate has not been reported in purine phosphoribosyltransferases. Crystals of protein tyrosine phosphatase 1b (PTP1b) complexed with a tight binding peptide revealed the structural mechanism responsible for the observed plasticity in substrate recognition. In addition, a series of actin metal nucleotide species revealed features of the reaction coordinate prior to the transition state.